27. A method according to claim 23 wherein IA protein is a variant IA protein

28. A method according to claim 23 wherein said IA protein is a non-naturally occurring IA protein.

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29. A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

a) receiving a protein backbone structure of an IA protein with variable residue positions;

b) selecting a set of variable positions;

c) establishing a group of potential rotamers for each of said variable residue positions;

d) analyzing the interaction of each of said rotamers in each group with all or part of the remainder of said protein to generate a library of IA proteins.--

REMARKS

Please cancel Claims 18-21 without prejudice or disclaimer as they are directed to non-elected inventions. Claims 22-29 are newly added. An Appendix of Pending Claims is attached for the Examiner's convenience.

Support for new claims 22 is found in Figure 3A and in the substitute specification at page 23, lines 23-25. Support fir new claims 23 and 24 is found in the substitute specification at page 12, line 32 through page 20, line 30. Support for new claims 25 and 28 is found in the substitute specification at page 32, lines 9-26. Support for new claim 26 is found in the substitute specification at page 27, lines 5-13. Support for new claim 27 is found in the substitute specification at page 43, lines 24-27. Support for new claim 29 is found in the substitute specification at page 45, lines 19-25 and at page 47, lines 12-22.

Applicants note that cancellation of the claims to the non-elected inventions does not change inventorship of the present invention.

Traversal of Additional Restriction Requirement

Applicants respectfully assert that the additional restriction requirement imposed by the Examiner is erroneous. Within the context of the claims, the election of species presently imposed makes it difficult, if not impossible, to obtain a claim as broad as

claim 1 which is directed to IA proteins that are less than 97% identical to the wild type insulin proteins. Moreover, such an additional restriction requirement is not in keeping with Applicant's invention.

Applicants respectfully submit that the Examiner has overlooked the underlying concept of Protein Design Automation (PDA™), the computational method upon which the invention is based. PDA™ is computational modeling system that allows the generation of extremely stable proteins without necessarily disturbing the biological function of the protein itself. This computational processing results in a set of optimized insulin activity proteins, i.e. a primary library of sequences predicted to exhibit desired physical and/or biochemical properties, such as modulated potency, increased hexmer formation, increased preference for the R state, increased binding affinity, increased stability and activity, etc. See the substitute specification at page 30, lines 4-28, page 32, lines 11-12, page 66, lines 24-27, and page 68, lines 33-34.

Generally speaking, this is done as follows. A three dimensional structure of a protein is input. Each residue position may be classified as a surface, boundary or core residue, and the residue positions are classified as either fixed or variable. For each variable position, a set of amino acid side chain rotamers are chosen, with at least one variable residue position having rotamers from at least two different amino acid side chains. The calculation then proceeds as follows: for each variable position, the energy of interaction of each rotamer with both the template (e.g. anything that is fixed, including the backbone and any fixed residues) and all possible rotamers at all variable positions is calculated. This is done using any number of different scoring functions. The calculation of the energy of interaction is facilitated by the use of Dead End Elimination (DEE). DEE is used to decrease the number of required calculations by eliminating rotamers that cannot be part of the global minimum; that is, by throwing out "bad" rotamers, the number of rotamers that needs to be analyzed decreases, and the global minimum solution is found more quickly. Once the global minimum is reached, local minima can be found by using additional analysis, such as a Monte Carlo analysis, which makes random changes and then recalculates the energy of interaction. Members of this library may then be chemically synthesized as described in the specification and used in biological assays, receptor binding assays, anti-viral and anti-proliferation assays to confirm that the sequence variants have the desired property.

As can be seen from the foregoing discussion, PDA™ avoids the problems associated with different mutagenesis strategies, such as random mutagenesis, point mutagenesis by error-prone PCR, cassette mutagenesis, and DNA shuffling, as all of these techniques require the actual synthesis of the mutated protein to determine whether or not a given variant has activity. In contrast, PDA™ generates a threshold or cutoff to eliminate disfavored sequences, i.e. those that lack the desired biological features, thereby increasing the percentage of useful variants that are synthesized.

As can be seen by the foregoing discussion, the claims are directed to a genus of variants that share common structural and functional properties. More importantly, the claims are directed to variants that have at least one and more preferably more than one substitution. Thus, the inventions are not "independent" as defined in M.P.E.P. § 802.01 and § 806.04(h). Applicants respectfully request withdrawal of the election of species requirement.

Attached hereto is a marked-up version of the changes made to the specification and claims by this Amendment. The attached page is captioned **"Version with markings to show changes made."**

The Examiner is invited to contact the undersigned at (415) 781-1989 if any issues may be resolved in that manner.

Dated: 6/17/02

Respectfully submitted,

DORSEY & WHITNEY LLP

By: _____
Renee M. Kosslak, Reg. No. 47,717 for
Robin M. Silva, Reg. No. 38,304
Filed under § 1.34(a)

Four Embarcadero Center - Suite 3400
San Francisco, California 94111-4187
Telephone: (415) 781-1989
1085216.RMK

"VERSION WITH MARKINGS TO SHOW CHANGES MADE"

In the Specification:

The paragraph beginning at line 17 of page 39 has been amended a follows:

Thus, in one preferred embodiment, PDA design is used to generate IA proteins that promote hexamer formation, but occlude phenol binding. In one aspect of this embodiment, IA proteins are generated that are stable and form hexamers in the absence of phenolic [oreservatives] preservatives. Some of these IA proteins may form hexamers that are more stable than the human insulin bound to a phenolic compound. In this embodiment, the PDB entry 1wav was chosen. For the PDA calculation, the entire insulin hexamer complex, consisting of 6 A-chains (chains 1, 3, 5, 7, 9, and 11 in hexamer) and 6 B-chains (chains 2, 4, 6, 8, 10, and 12 in the hexamer) was used.

In the claims:

Claims 18-21 have been cancelled.

Please amend claim 1 as follows:

1. (Amended) A non-naturally occurring insulin activity (IA) protein comprising an amino acid sequence which comprises substitution of at least one amino acid residue when compared to an amino acid sequence of a naturally occurring human insulin and wherein said IA protein has an altered property when compared to the same property of human insulin and binds to a cell comprising an insulin receptor.

Please amend claim 10 as follows:

10. (Amended) The non-naturally occurring IA protein according to claim 1 wherein said IA protein comprises an amino acid sequence selected from the group of amino acid sequences shown in Figure 3A (SEQ ID NO: 7), Figure 3B (SEQ ID NO: 8), Figure 3C (SEQ ID NO: 9), Figure 3D (SEQ ID NO: 10), Figure 3E (SEQ ID NO: 11), Figure 3F (SEQ ID NO: 12), Figure 3G (SEQ ID NO: 13), Figure 4A (SEQ ID NO: 14), Figure 4B (SEQ ID NO: 15), Figure 4C (SEQ ID NO: 16), Figure 4D (SEQ ID NO: 17), Figure 4E (SEQ ID NO: 18), Figure 4F (SEQ ID NO: 19), Figure 4G (SEQ ID NO: 20), Figure 5A (SEQ ID NO: 21), Figure 5B (SEQ ID NO: 22), and Figure 5C (SEQ ID NO: 23).

APPENDIX OF PENDING CLAIMS

1. (Amended) A non-naturally occurring insulin activity (IA) protein comprising an amino acid sequence which comprises substitution of at least one amino acid residue when compared to an amino acid sequence of a naturally occurring human insulin and wherein said IA protein has an altered property when compared to the same property of human insulin and binds to a cell comprising an insulin receptor.

2. A non-naturally occurring IA protein according to claim 1, wherein said IA protein comprises a substitution at a position selected from the group consisting of positions A3, A5, A6, A7, A11, A15, A16, A19, A20, B2, B7, B15, B19, and B22.

3. A non-naturally occurring IA protein according to claim 2, wherein substitution is selected from the group of A7-S, A7-E, B2-E, B2-T, B4-Y, B7-Y, B4-F, B7-Y, B7-E, and B7-D.

4. A non-naturally occurring IA protein according to claim 1, wherein said IA protein comprises substitutions of at least four amino acid residues.

5. A non-naturally occurring IA protein conformer having a three dimensional backbone structure that substantially corresponds to the three dimensional backbone structure of human insulin, wherein the amino acid sequence of said conformer and said amino acid sequence of said human insulin are less than about 98% identical.

6. A non-naturally occurring IA protein comprising at least one amino acid substitution as compared to human insulin, wherein at least one of said substitutions is selected from the amino acid residues at positions selected from positions B5 and B14, and wherein said IA protein forms a hexamer in the absence of a phenolic preservative.

7. The non-naturally occurring IA protein according to claim 6, wherein said IA protein comprises a substitution selected from the group of B5-F, B5-W, B14-F, B14-W, B14-Y, and B14-I.

8. The non-naturally occurring IA protein according to claim 1, wherein said IA protein comprises At least 5 substitution at positions selected from the group consisting of positions A1, A10, A16, A17, A19, B1, B2, B4, B8, B11, B12, B14, B25, B26, B27 and B28.

9. The non-naturally occurring IA protein according to claim 8, wherein said substitutions are selected from the group of substitutions consisting of A1-N, A10-Q, A16-Y, A17-Y, A19-F, B1-D, B2-K, B4-F, B8-L, B11-I, B12-R, B14-W, B25-N, B26-F, B27-D, and B28-N.

10. (Amended) The non-naturally occurring IA protein according to claim 1 wherein said IA protein comprises an amino acid sequence selected from the group of amino acid sequences shown in Figure 3A (SEQ ID NO: 7), Figure 3B (SEQ ID NO: 8), Figure 3C (SEQ ID NO: 9), Figure 3D (SEQ ID NO: 10), Figure 3E (SEQ ID NO: 11), Figure

3F (SEQ ID NO: 12), Figure 3G (SEQ ID NO: 13), Figure 4A (SEQ ID NO: 14), Figure 4B (SEQ ID NO: 15), Figure 4C (SEQ ID NO: 16), Figure 4D (SEQ ID NO: 17), Figure 4E (SEQ ID NO: 18), Figure 4F (SEQ ID NO: 19), Figure 4G (SEQ ID NO: 20), Figure 5A (SEQ ID NO: 21), Figure 5B (SEQ ID NO: 22), and Figure 5C (SEQ ID NO: 23).

11. A recombinant nucleic acid encoding the non-naturally occurring IA protein of claim 1 or 10.

12. An expression vector comprising the recombinant nucleic acid of claim 11.

13. A host cell comprising the recombinant nucleic acid of claim 11.

14. A host cell comprising the expression vector of claim 12.

15. A method of producing a non-naturally occurring IA protein comprising culturing the host cell of claim 13 under conditions suitable for expression of said nucleic acid.

16. The method according to claim 15 further comprising recovering said IA protein.

17 A pharmaceutical composition comprising an IA protein according to claim 1 or 10 and a pharmaceutical carrier.

22. A non-naturally occurring IA protein according to claim 10 wherein said IA protein comprises the amino acid sequence shown in Figure 3A (SEQ ID NO: 7).

23. A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

a) receiving a protein backbone structure of an insulin activity (IA) protein with variable residue positions;

b) selecting a set of variable positions;

c) establishing a group of potential rotamers for each of said variable residue positions;

d) analyzing the interaction of each of said rotamers in each group with all or part of the remainder of said protein to generate a set of optimized protein sequences.

24. A method according to claim 23, wherein said analyzing step further comprises the use of at least one scoring function.

25. A method according to claim 23 wherein said IA protein is a wild-type IA protein.

26. A method according to claim 23 wherein said wild-type IA protein is a mammalian insulin species selected from the group consisting of bovine (GenBank accession number IPBO), dog (GenBank accession number IPDG), sheep (GenBank accession number INSH), cat (GenBank accession number INCT), pig (GenBank accession number IPPG), mouse (GenBank accession numbers INMS1 and INMS2), rat (GenBank accession numbers IPRT1 and IPRT2), horse (GenBank accession number IPHO), rabbit (GenBank accession number

INRB), guinea pig (GenBank accession number IPGP), hamster (GenBank accession number INHY), goat (GenBank accession number INGT), chimpanzee (GenBank accession number A42179), and green monkey (GenBank accession number B42179).

27. A method according to claim 23 wherein IA protein is a variant IA protein

28. A method according to claim 23 wherein said IA protein is a non-naturally occurring IA protein.

29. A method executed by a computer under the control of a program, said computer including a memory for storing said program, said method comprising the steps of:

a) receiving a protein backbone structure of an IA protein with variable residue positions;

b) selecting a set of variable positions;

c) establishing a group of potential rotamers for each of said variable residue positions;

d) analyzing the interaction of each of said rotamers in each group with all or part of the remainder of said protein to generate a library of IA proteins.